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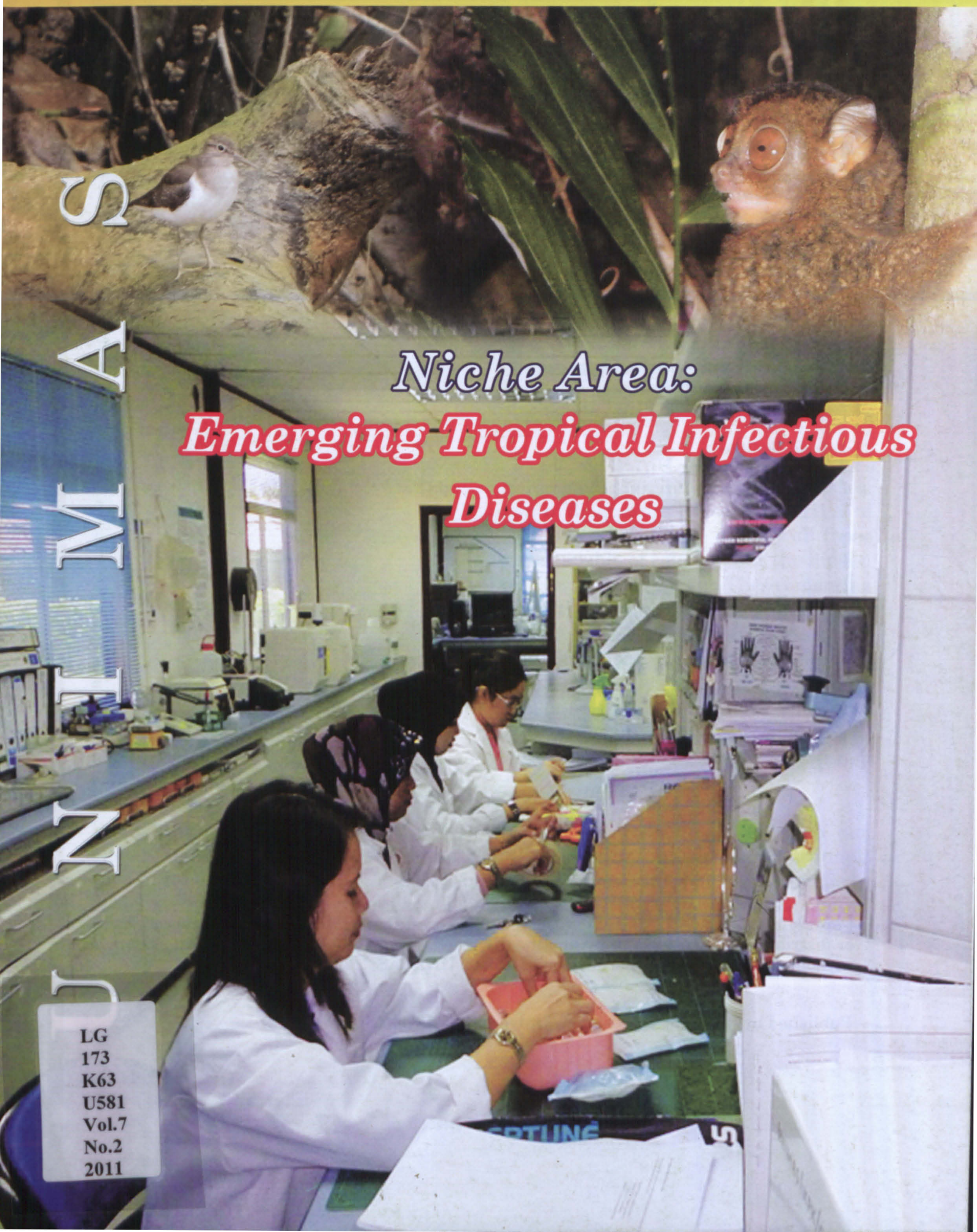

Niche Area: Emerging Tropical Infectious Diseases

Editorial Committee

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FOREWORD

It is my pleasure to welcome you to the December issue of Research Update. This issue highlights another UNIMAS research niche area, which is "Emerging Tropical Infectious Diseases" that provides insights for us to understand their emergence and their impacts. International collaboration on emerging diseases has been gaining momentum and support in recent years.

Zoonoses, or zoonotic diseases have been in existence since early historical times. These diseases are caused by infectious agents that are transmissible under natural circumstances from vertebrate animals to humans, either from wild or domestic or from products of animal origin. Certain types of zoonoses, such as rabies and malaria, are well known to the general public, however, it is understood that a substantial number of lesser-known zoonoses exist in other pockets of the world. It is believed that there are undoubtedly vast numbers of zoonoses that have the potential to cause serious damage to the public health if introduced into humans.

There are obvious gaps in the global understanding of emerging infectious diseases. The need for more research in the development of diagnostics for early detection of these diseases is crucial and challenging. The greatest challenge pose by all these emerging diseases is not what we know they are capable of causing, but the hidden damaging possibilities that these diseases may caused in the future. These emerging infectious diseases pose major risks to us as well as the animal populations as a whole. We are directly at risk from infection and indirectly at risk through our food supply. These diseases, whose incidence in humans have increased in the past two decades and threaten to increase in the near future, are perennially challenging our public health. Through research, we can rise to these challenges effectively and efficiently. It is imperative that continuous research and development ensure new advances in science and medicine in helping us in our battle against these emerging infectious diseases.

The research projects presented in this issue will undoubtedly enhance our understanding on the infectious diseases and their impacts, and ultimately, triumph our continuous battle against them. It is hope that the ultimate goal for research is to enable us to predict future emergence of diseases. To our researchers who contributed to this issue, it is my sincere best wishes that answers to the questions posed by these diseases can be found through your noble research endeavours.

Prof Dr Peter Songan
Deputy Vice Chancellor (Research & Innovation)
Universiti Malaysia Sarawak

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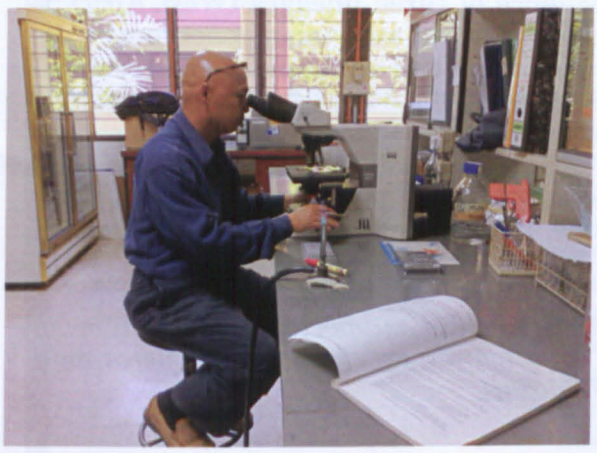
THE PREVALENCE OF LEPTOSPIROSIS IN THE REJANG BASIN OF SARAWAK

Researchers: *Lela Suut, Haironi Yusof, M.Taha Arif, Nor Aliza A. Rahim, Joseph Tau Katip and M. Raili Suhaili*
Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak

Bintangor clinic

Kapit Leptospirosis survey



Leptospirosis laboratory work

Leptospirosis is a zoonotic infection that has been recognised as a reemerging infection due to its increasing incidence in both developing and developed countries. This infection is caused by the pathogenic microorganism *Leptospira interrogans*. This paper aims to study the prevalence of leptospirosis infection among the human population living in the Rejang basin of Sarawak and its pattern of distribution. Human sera was collected from 363 individuals aged between 5-80 years (with consent, at random), who lived in towns and small settlements along the Rejang river and its tributaries. ELISA tests were performed on the sera using the Panbio Leptospira IgM ELISA kit according to the manufacturer's protocol. Suggestive evidence of leptospiral infection was detected in 111 individuals (30.6%) with 77 cases recording strong positivity results, whilst the remaining was equivocal. Amongst the seropositive cases, 67.5% were females. The median age for this group was 37.0 years (age range 9-80 years old) and majority were farmers and housewives (68.9%). Almost half of the positive cases (48.0%) admitted to having symptoms of fever about 2-4 weeks prior to the sampling, and 41.6% admitted to having >4 times outdoor activities per week. Farming and water activities were the main outdoor activities in 54.5% of seropositive cases. Majority of the positive cases (80.5%) also cited using natural water source (river, rain water) as their main water supply. More than half of the positive cases (53.3%) were found in Nanga Merit and the rest were scattered in other settlements. In the equivocal group, the median age was 38 years old, with more than half were farmers and housewives (55.9%). They also reported fever as one of the symptoms experienced prior to sampling and also had frequent outdoor activities (35.3%) that included collection of jungle produce, farming as well as water activities. Within this group, usage of natural water source as primary water supply was reported in 76.5% of cases. These preliminary findings seem to suggest that there is a close association between the sample population daily activities (farming, water activities) and their water source with IgM leptospira positivity. These results will be further confirmed using leptospiral microscopic agglutination test (MAT).

AN OUTBREAK OF CHIKUNGUNYA VIRUS IN SARAWAK IN 2009

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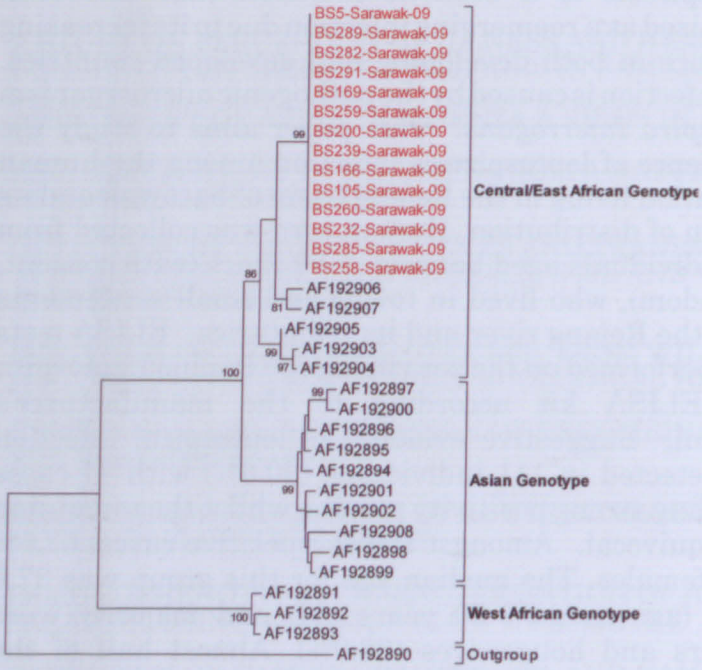
[2] Sarawak General Hospital



Phylogenetic analysis of CHIKV partial E1 gene nucleotide sequences. Reference CHIK sequences were obtained from GenBank and are indicated by their respective accession numbers. All Sarawak CHIKV sequences are indicated in red. Bootstrap values > 80% are indicated at selected nodes. The scale at the bottom refers to the evolutionary distances used to infer the phylogenetic tree.

In 2009, Sarawak experience an outbreak of the Chikungunya virus (CHIKV) that was presented as an acute infection of abrupt onset, characterized by high fever, arthralgia, myalgia, headache, and rash. The Institute of Health & Community Medicine (IHCM) in Universiti Malaysia Sarawak was invited by the Sarawak public health authorities during the initial onset of the outbreak to help investigate the etiologic cause of the outbreak which at that time was unknown and occasionally mistaken as a dengue outbreak. Patient samples were collected and sent to IHCM for virus identification and molecular typing. CHIKV is a mosquito-transmitted alphavirus belonging to the family Togaviridae, with an envelope and single-stranded positive-sense RNA genome. The virus is transmitted mainly from human to human by the bite of the *Aedes* mosquito, primarily *Aedes aegypti*. First isolated in 1952-53 in Africa, CHIKV outbreaks have since been recorded around the world. The first recorded outbreak in Asia was in Bangkok in 1958 followed by a number of outbreaks in other asian countries. In Malaysia, CHIKV was first recorded in peninsular Malaysia in 2007. Molecular typing of CHIKV has generally been associated with phylogenetic analysis of the structural E1 gene region of the virus genome. Phylogenetic analysis of this region has shown the prevalence of three lineages (West African, Central/East African and Asian genotypes) with distinct genotypic and antigenic characteristics. In the Sarawak 2009 outbreak, CHIKV isolated from patient samples sent to IHCM, were shown to be phylogenetically associated with the Central/East African genotype (Figure). To our knowledge, this is the first recorded instance of CHIKV in Sarawak.

This research was supported by Institute of Health & Community Medicine's operational research budget.

STUDIES TO DETERMINE THE EVOLUTIONARY HISTORY AND THE SOURCE OF HUMAN INFECTIONS WITH THE MALARIA PARASITE *PLASMODIUM KNOWLESI*

Researchers: *Kim-Sung Lee[1], Paul C.S. Divis[1], Siti Khatijah Zakaria[1], Asmad Matusop[2], Roynston A. Julin[1], David J. Conway[3], Janet Cox-Singh[1,4] and Balbir Singh[1]*

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Long-tailed macaque being tagged with a microchip after blood sampling and before release.

Malaria in humans was thought to be caused by four species of *Plasmodium* (*P. falciparum, P. vivax, P. malaria* and *P. ovale*) until a team of researchers from UNIMAS described a large number of human infections with *Plasmodium knowlesi* in the Kapit Division of Sarawak in 2004. Human infections have been subsequently described in many countries in Southeast Asia, leading to the recognition of *P. knowlesi* as the fifth cause of human malaria. Long-tailed and pig-tailed macaques, the most common non-human primates in Southeast Asia are the natural hosts of *P. knowlesi*. There had been no documented evidence of *P. knowlesi* or any other malaria parasites in monkeys in Sarawak, and although a monkey source for the hundreds of human *P. knowlesi* infections that have been described in the Kapit Division was likely, it remained to be proven. Therefore a study was initiated in collaboration with the Sarawak Health Department, the London School of Hygiene & Tropical Medicine and St George's University of London to examine monkeys in the Kapit Division for malaria parasites and to compare the molecular identity of *P. knowlesi* derived from monkeys and humans. A total of 108 wild monkeys were studied from 17 different locations in the Kapit Division. Five species of *Plasmodium* (*P. knowlesi, P. inui, P. cynomolgi, P. fieldi* and *P. coatneyi*) were detected in the monkeys, with an extremely high prevalence of *P. inui* and *P. knowlesi*. Following DNA sequence analysis of the circumsporozoite gene and the mitochondrial genome, it was found that the number of *P. knowlesi* genotypes per infection was much higher in monkeys than humans, some genotypes were shared between the two hosts and no major genotypes were associated exclusively with either host. This strongly indicated that monkeys are the reservoir hosts for *P. knowlesi* in the Kapit Division. Analyses of the mitochondrial genome sequence data indicated that *P. knowlesi* existed in monkeys prior to human settlement in Southeast Asia and underwent a recent population expansion approximately 30,000-40,000 years ago. Thus, humans were infected with these parasites from the original and reservoir monkey hosts probably since humans first entered the forests of Southeast Asia. The studies therefore indicate that human infections with *P. knowlesi* are not newly emergent in Southeast Asia and that knowlesi malaria is primarily a zoonosis with wild monkeys as the reservoir hosts. However, it is possible that ongoing ecological changes resulting from deforestation, with changes in mosquito behavior and an associated increase in the human population, could enable this species of *Plasmodium* to switch to humans as the preferred host.

A FOLLOW UP STUDY OF LIPID PROFILE AND FASTING GLUCOSE LEVELS AFTER FIVE YEARS IN SIBU, SARAWAK

Researchers: *Haironi Yusoff and Annuar Rapaee*
Faculty of Medicine and Health Sciences, University Malaysia Sarawak

CVS 5 year follow up study.

In order to determine the changes in lipid profile and fasting glucose levels in an urban community, a community-based epidemiological survey was conducted to compare 5-year mean changes in lipid and glucose levels after intervention. Sample size was 181 respondents with 66 (36%) males and 115 (63.5%) females. The majority of respondents were Malays (95%) with a mean age of 48.4 years (SD±11.7). Only 19 (15.5%) had family history of cardiovascular disease and 8 (4.4%) respondents had cardiovascular disease. Of the sampled population, 96 (53%) were obese, 41 (22.7%) were hypertensive and 30 (16.6%) had diabetes. Calculation of the cardiovascular risk factors using the Framingham Score showed that 121 (66.9%) of the respondents had moderate to high risk. Mean levels for LDL-cholesterol and triglyceride was higher whilst HDL-cholesterol was lower in the initial survey ($p<0.05$). Fasting glucose levels was noted to be higher five years after follow-up ($p<0.05$). Comparison between the cardiovascular risk ratio between before and after 5 years of follow-up showed a higher level in the initial group ($p<0.05$). There was no difference in total cholesterol levels between the two groups. The study indicates significant degree of improvement in lipid profile but worsening levels of fasting glucose in the community, which is consistent with the increase trends of diabetes.

DISEASE PATTERN IN LYMPH NODE BIOPSY: AN EXPERIENCE IN A SARAWAK REFERRAL DIAGNOSTIC LABORATORY

Researchers: *Mohammad Zulkarnaen bin Ahmad Narihan[1], Dayangku Norlida Awang Ojep[1], Zainal Abidin Rahim[1], Henry Rantai Gudum[1] and Jacqueline Wong Oy Leng[2]*
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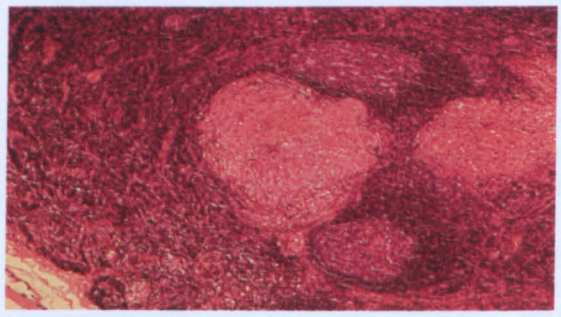


Hematoxylin and eosin staining showing a lymph node biopsy with chronic granulomatous lymphadenitis.

Excision biopsy of the lymph nodes is commonly performed for the investigation of patients with lymphadenopathy. A study based on the Sarawak population would provide data that reflects on the pattern of diseases in lymph nodes encountered by clinicians in a local setting. This study was performed to determine the pattern of lymph node pathology seen in the pathology department of a tertiary-care hospital in Sarawak. A total of 176 lymph node biopsies for the investigation of lymph node enlargement were retrieved from the files in a diagnostic histopathology laboratory from the year 2007 to 2009. The majority of the cases were in the age range of 21 to 60 years with a mean age of 43.77 years. The pathology consisted of 27.8% malignant lymphomas, 17.1% metastatic carcinomas, 24.4% reactive hyperplasia, 24.4% chronic granulomatous lymphadenitis, 1.1% metastatic melanoma, and 11.8% other diagnoses (necrosis, abscess, necrotizing lymphadenitis favouring SLE, toxoplasma lymphadenitis, Kikuchi's disease and Rosai Dorfmann disease). The most common site of biopsy was from the head and neck region, particularly the cervical group of nodes (n=111, 63.1%) followed by the inguinal region (11.9%). The finding of malignancy reached more than 60% of the nodal biopsies in the middle age groups and the elderly age groups. Our study showed that the disease pattern in lymph node biopsies encountered in Sarawak General Hospital's histopathology unit did not differ significantly from those seen West Malaysia. Our study concluded that the most common nodal pathology seen in the pathology department of a tertiary-care hospital in Sarawak was malignant lymphomas, followed closely by chronic granulomatous lymphadenitis.

A STUDY ON PERCEPTION OF ILLNESS AND HEALTH SEEKING BEHAVIOUR AMONG COMMUNITY IN SELECTED VILLAGES IN SAMARAHAN DISTRICT

Researchers: *Haironi Yusoff, Zainab Tambi, Aye Aye Aung and Khatijah Yaman*
Faculty of Medicine and Health Sciences, University Malaysia Sarawak

Survey conducted in the kampongs together with Year 1 Medical Students.

Survey and health screening.

In order to determine the health seeking behaviour and perception of illness among 4 selected villages in Samarahan, a cross-sectional study was conducted. The selected villages were Kampung Tanjong Bundong (39), Kampung Baru (39), Kampung Niup (41) and Kampung Tanjong Parang (38). Perception score was calculated based on a questionnaire consisting of 11 questions, ranging from balanced diet, physical activity, immunization, antenatal follow-up, pap smear, breast self examination, promiscuity, alcohol intake, substance abuse, cigarette smoking and perception on general health. The second part of the questionnaire was on health seeking behaviour on traditional medicine practices, vitamin intake, treatment preference and beliefs. Results showed that 76 respondents (48.4%) preferred government health centres and 48 (30.6%) respondents self-medicate while others preferred going to private clinics, pharmacy or traditional medication. The majority of the respondents (108) took traditional medicine comprising of herbal medicine (36.3%), medicinal oil (15.3%) or medicinal plants (14%). Of the total, 89 respondents (56.7%) believed that illness is due to causes other than microorganisms. A total of 121 (77.1%) respondents believed that seeking health treatment is only necessary when one is unwell. When the perception scores were tabulated, 104 respondents (66.2%) had good perception on health. It was noted that those taking vitamin supplements had better perception than those that did not take vitamins (χ=9.81, df = 1, p=0.002). Males were also noted to be more likely to believe that illness is due to causes other than microorganisms. Results of this study may help to shed some light on improving health seeking behaviour among the community. This is because although the perception of health may be good, their health seeking behaviour may still be affected by cultural influence of traditional beliefs and practices.

ASSESSMENT OF AVIAN INFLUENZA A VIRUS IN FOREST BIRDS IN MALAYSIA

Researchers: *Zahirunisa Abd Rahim, Ismail Ahmad and Mustafa Abdul Rahman*
Faculty of Resource Science and Technology, Universiti Malaysia Sarawak

Taking sample from bird's throat.

Maroon woodpecker (*Dinopium rafflesii*).

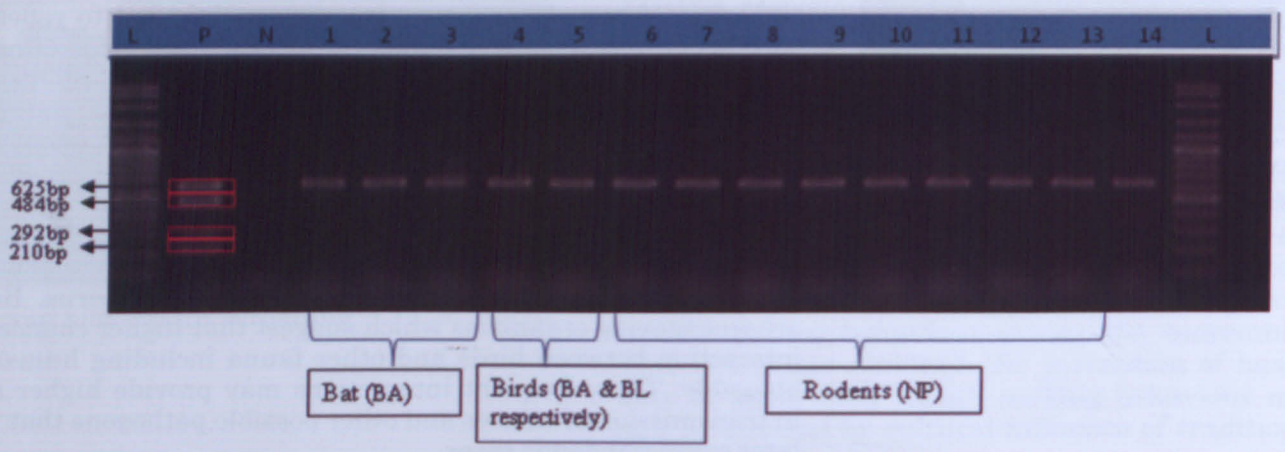
Emerging infectious diseases can be defined as infections that have appeared recently or existed previously but demonstrate frequent incidence as well as expanding the introduction of the infections to other geographical areas. These diseases are transmitted into the environment via a number of transmission routes. Avian influenza A virus is an example of an important emerging infectious disease. The transmission cycle of this virus is very much influenced by the frequency of interactions between infected migratory wild birds and residential wild birds. The transmission occurs when wild birds occupying different habitats interacts in the interphase where they meet. Wild birds are known to be the reservoir for avian influenza A virus. However, the hosts range from migratory wild birds to forest birds have not been fully elucidated with avian influenza A disease. This study focuses on two major components; (1) to detect the occurrence of avian influenza virus in different habitat types and (2) to elucidate the potential hosts for avian influenza A virus in Malaysia. Seventeen study sites in Malaysia were selected including Balambangan Island (Sabah), Sg Dusun Wildlife Reserve, Fraser's Hill Forest Reserve, Tasek Bera Ramsar Site, Krau Wildlife Reserve (peninsular Malaysia), and Buntal, Batang Ai National Park, Sibu (Bukit Lima Forest Park, Bukit Aup Recreational Park and Ta Ann Naman plantation), Lanjak Entimau Wildlife Sanctuary (Sg Menyarin and Sg Bloh), Belaga (Sg Asap: Strip 1 and Strip 2), Kapit (Nanga Merit: orchard and forest) and Mulu National Park (Sarawak). These areas were grouped into seven habitat types categorized as primary forest, secondary forest, urban, monoculture, mixed forest of lowland and limestone, beach forest and mixed forest of secondary growth and orchard to reflect a portion of the vast tropical habitat. A total of 2,219 virus samples were isolated from 1,134 birds and amplified based on reverse transcriptase polymerase chain reaction (RT-PCR) and also immunocapture RT-PCR. All samples were treated using H5, H6, H7, H9 and nucleoprotein (NP) primers. All samples tested negative by RT-PCR, suggesting perhaps, that birds in Malaysia are free from avian influenza A virus. To date there are no critical reviews conducted to discriminate the host of influenza and the habitats that may lead to the transmission of the virus. Birds are free moving organisms which suggest that higher chances of interaction between birds and other fauna including human is plausible. Thus, frequent interactions may provide higher risk in transmission of viruses and other possible pathogens that will later cause pandemic cases.

ESCHERICHIA COLI IN WILDLIFE FROM A NATURAL AND DISTURBED HABITATS OF SARAWAK, EAST MALAYSIA

Researchers: *Kasing Apun, Kho Kai Ling, Lesley Maurice Bilung, Mohd. Tajuddin Abdullah, Mustafa Abdul Rahman and Chen Yik Ming*
Faculty of Resource Science and Technology, Universiti Malaysia Sarawak

Wildlife is well-known to be involved in most of the zoonotic diseases. The potential for dissemination of pathogenic enteric bacteria by wildlife to human is of concern due to their ability to cause epidemic and pandemic human diseases. Destruction or disturbances of the wildlife habitat have also been identified as a factor that leads to the emergence of these zoonoses outbreaks. Therefore, this study was performed to compare the occurrence of *E. coli* and to detect *E. coli* O157:H7 in wildlife hosts comprising of birds, bats and rodents from natural and disturbed habitats in Sarawak, Malaysia. Disturbed habitats comprised of two urban forests, an oil palm plantation habitat located in Sibu and a human settlement area in Nanga Merit. A forest area located in Nanga Merit, Kapit represented the case for a natural habitat. The swabs samples collected from selected wildlife were tested for the occurrence of *E. coli* on selective agar and standard biochemical tests. Multiplex PCR assay for genes encoding the Shiga-toxin (*Stx1, Stx2*), O157 antigen (*rfbE*) and H7 antigen ($fliCh_7$) were performed for the detection of *E. coli* O157:H7. Subsequently, representative *E. coli* isolates were selected to study the genetic profiles by using ERIC-PCR and PFGE. The occurrence of *E. coli* was consistently higher in rodents followed by birds and bats regardless of the type of habitats. Genes encoded for Stx1, Stx2 and rfbE were not detected during the screening of *E. coli* O157:H7. However, the $fliCh_7$ gene was detected in 27 *E. coli* isolates. Sequence analysis showed that 4 *E. coli* isolates from Jubilee Park, Sibu were associated with extraintestinal pathogenic *E. coli*. Comparison of the genetic relatedness among the *E. coli* isolates based on the ERIC and PFGE genetic fingerprinting patterns showed that all of the *E. coli* isolates were genetically heterogeneous. They were arbitrarily grouped within the dendrogram regardless of the hosts and habitats. The results indicated that both ERIC-PCR and PFGE have limited application in the genotyping of *E. coli* strains isolated from wildlife and environment. These results suggest that wildlife in Sarawak do not served as an important reservoir of *E. coli* O157:H7. However, precautions have to be taken as extraintestinal pathogenic *E. coli* may pose a zoonotic risk for humans and other animals. Both genotyping methods indicated high degree of diversity within *E. coli* isolated.

This research was supported by research grant no.: E14006/F07/06/ZRC/03/2007(3) and FRGS/01(19)/748/2010(34).



Positive Amplicon samples of *E. coli* with $Flich_7$ gene.

DETECTION OF VIRULENCE GENES AND TRANSMISSION OF *SHIGELLA, SALMONELLA* AND *YERSINIA* SPP. FROM WILD ANIMALS, SOIL AND WATER FROM DISTURB AND NATURAL HABITATS IN SARAWAK

Researchers: *Chen Yik Ming, Kasing Apun, Lesley Maurice Bilung, Hashimatul Fatma Hashim, Mohd. Tajuddin Abdullah, Kho Kai Ling and Adom Benjamen*
Faculty of Resource Science and Technology, University Malaysia Sarawak

Bat sampling.

Shigella spp., *Salmonella* spp. and *Yersinia* spp. are gastrointestinal pathogens which infect avians, bats, rodents and other small mammals. Anal or cloacal swabs, feacal and small intestinal of avians, chiropterans and rodents were collected from human settlement area (Site 1) and pristine forest area (Site 2) in Nanga Merit, Kapit, Sarawak. Soil and water samples were collected along the trapping routes at both sites. Xylose Lysine Decarboxylase (XLD) agar and Cefsulodin-Irgasan-Novobiocin (CIN) agar were used for the isolation of *Shigella* spp., *Salmonella* spp. and *Yersinia* spp. Standard biochemical tests were performed for the identification of bacteria. Through multiplex PCR, virulence gene (*ipaH* and *ial*) of *Shigella* spp. was detected in three chiropterans and one avian sample from human settlement and one avian, two chiropterans, a soil and a water sample from the forest. A chiropteran isolates from both sites were positive for *Flic* gene which is a virulence gene in *Salmonella typhimurium*. No *Yersinia* spp. was found during isolation.

This research was supported by research grant no.: E14006/F07/06/ZRC/03/2007(3).

ISOLATION OF *CAMPYLOBACTER* SPP. FROM WILD BIRDS, RODENTS AND BATS IN A DISTURBED AND UNDISTURBED ECOLOGICAL HABITATS IN KAPIT, SARAWAK, MALAYSIA

Researchers: *Adom Benjamen, Kasing Apun, Lesley Maurice Bilung and Mustafa Abdul Rahman*
Faculty of Resource Science and Technology, Universiti Malaysia Sarawak

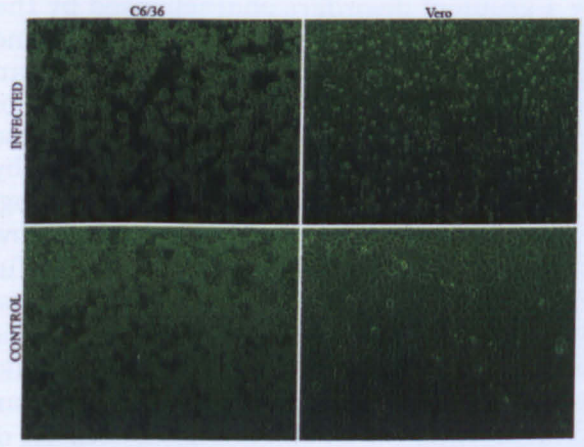
Sample A2776, Post Enrichment on CCDA Agar, 48hr, 37°C, microaerophilic atmosphere (Campygen, Oxoid CN25, U.K.).

The prevalence of *Campylobacter* spp among wild birds, rodents and bats were investigated in disturbed and undisturbed habitats in Kapit, Sarawak using conventional direct plating and a selective enrichment method combined with PCR as a confirmatory identification tool. Birds were trapped using mist nets installed at several locations and were checked every two hours. Sterile cotton swabs were inserted into the cloacal area. Alternatively, fresh faeces were sampled aseptically. Rodents were trapped using cage traps, and anal swab were collected aseptically. Similarly, cloacal swab were collected from bats trapped using mist-nets and harp traps. Swab were transferred into tubes containing 1.33% Cary-Blair media which were then inoculated onto selective CCDA agar (Campygen, Oxoid, U.K) on the same day. All growth were observed for their morphology and tested for oxidase reactivity. Greyish colonies which were oxidase positive or weakly positive were considered as presumptive for *Campylobacter* and subjected to Gram stain and biochemical tests (BBL Crystal) where necessary. Out of 256 samples tested, 175 (68.4%) samples showed positive growth of which 20 (7.8%) isolates were presumably identified as *Campylobacter* after enrichment in Bolton Selective enrichment broth. However, further biochemical tests showed none of these could be grouped as *Campylobacter*. Further test using specific-PCR confirmed none of these isolates belong to *Campylobacter*. These results indicate that the wildlife studied here do not harbor any *Campylobacter* species.

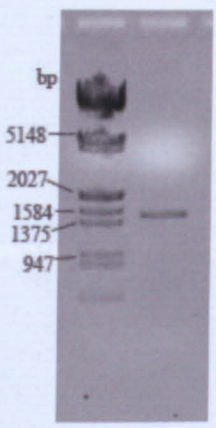
This research was supported by research grant no.: E14006/F07/06/ZRC/03/2007(3).

EXPRESSION AND PURIFICATION OF RECOMBINANT CHIKV E1 PROTEIN AS A REAGENT FOR DIAGNOSTIC ASSAYS

Researchers: *Magdline S.H. Sum and David Perera*
Institute of Health and Community Medicine, Universiti Malaysia Sarawak



A.



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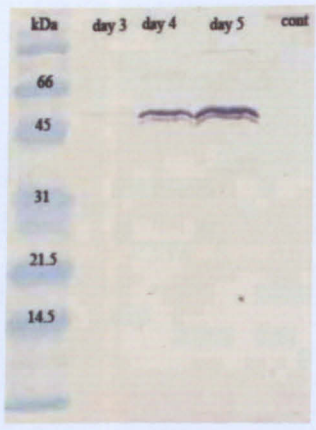

C.

CHIKV was propagated in two cell lines. Infected and control C6/36 and Vero cells (A). Amplification of the E1 gene (B). Expression of recombinant protein in Sf9 insect cells (C).

Chikungunya virus (CHIKV) is an important human pathogen that causes a disease syndrome characterized by fever, headache, rash, nausea, vomiting, myalgia and arthralgia. The disease is caused by CHIKV, an alphavirus of the family *Togaviridae*. CHIKV is transmitted to humans by mosquitoes of the genus *Aedes*, particularly *Aedes aegypti* and *Aedes albopictus*. Though generally a non-fatal condition, the clinical illness is often associated with prolonged morbidity, which can impose enormous social and economic disadvantages on affected communities. CHIKV infections though rarely, may also be associated with complications such as encephalopathy and hepatic failure. Currently there are no specific treatments for CHIKV infections and no licensed vaccine for any alphavirus is available for human use. Enzyme linked immunosorbent assay (ELISA) and Reverse transcriptase polymerase chain reaction (RT-PCR) have been used as serological and molecular tools for the specific detection of CHIKV in patient samples. Though RT-PCR provides early and accurate diagnosis, its sensitivity for detection of CHIKV RNA in primary clinical specimens is sometimes limited particularly in cases where the sample viral load is low. An alternate strategy is to use serological methods in which CHIKV specific antibodies are detected using native viral antigen. Since high risk factors are associated with the extraction and purification of total antigen from live viruses, recombinant antigens have been used instead for rapid and specific serological detection. Hence the objective of the study was to develop an effective method to express and purify recombinant E1 protein as an alternative antigen. The baculovirus insect cell expression system was used for this purpose. This production system is a safe and efficient way of expressing proteins in large amounts and on a large scale in eukaryotic cells. Furthermore, protein expression in insect cells allows post-translational modifications, accurate folding and efficient secretion. This recombinant protein may be used as recombinant antigen in the development of a diagnostic assay for the detection of anti-CHIK antibodies in patient sera.

This research was supported by Institute of Health & Community Medicine's operational research budget.

BLAST CHARACTERISATION OF ACUTE MYELOID LEUKAEMIA (AML) IN SARAWAK BY 4-COLOUR FLOW CYTOMETRY

Researchers: *Tay Siow-Phing[1], Lau Lee-Gong[2], Lela Suut[1], Mohamad Razif Othman[1], Ong Gek-Bee[3], Chew Lee-Ping[3] and Henry Rantai Gudum[1]*

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[2]Normah Medical Specialist Centre

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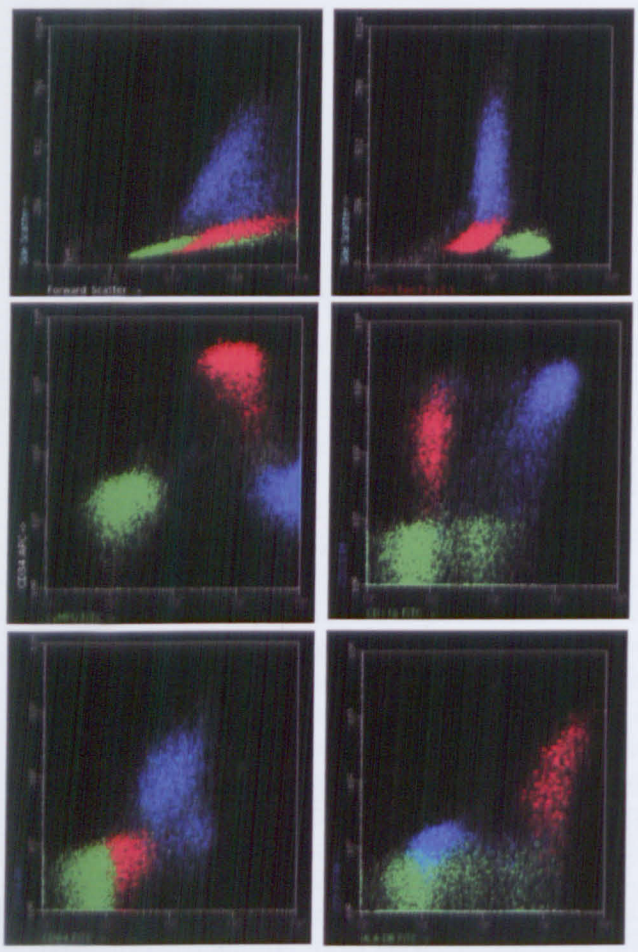

The identification of leukaemic blasts (red) was based on the cell size (forward scatter), cellular granularity (side scatter), CD45 and CD34 expressions, as compared to normal lymphocytes (green) and normal granulocytes (blue). Further characterisation of myeloid blasts was determined by the expression of specific myeloid markers (e.g. cytoplasmic MPO, CD13, CD11b, CD14, CD64, CD117, HLA-DR).

Leukaemias are a group of disorders characterised by the accumulation of abnormal white blood cells in the bone marrow. Multiparameter flow cytometry (FCM) plays an increasingly important role in the laboratory diagnosis of acute myeloid leukaemia (AML), particularly when precise lineage of the leukaemic blast cells cannot be defined by light microscopic morphology and cytochemistry staining. Currently, immunophenotypic analysis of the bone marrow cells by multiparameter FCM has also become a powerful tool for post-treatment monitoring of AML. This cross-sectional and experimental study aimed to characterise the blast populations of AML cases diagnosed in Sarawak. The bone marrow aspirates were first screened using an acute leukaemia screening panel consisting of 3 tubes of 4-colour markers to determine the lineage. Once myeloid lineage was confirmed, further blast characterisation was achieved using the AML panel consisting of 8 tubes of 4-colour markers. A total of 95 cases of AML were diagnosed from 2008-2010. There were 75.8% of adults and 24.2% of paediatric cases with male to female ratio of 1.3:1. Morphologically, the breakdown of AML cases according to French-American-British (FAB) classification was as follows: M0 – 1, M1 – 16, M2 – 38, M3 – 2, M4 – 14, M5 – 9, M6 – 3, M7 – 3 and unclassified – 9. The blast populations of 96.9% of the cases expressed CD45 dimly, 75.0% and 84.4% were positive for CD34 and cytoplasmic myeloperoxidase (MPO) respectively. The expression of the other markers was as follows: CD33 – 91.7%, CD13 – 86.5%, CD117 – 85.4%, HLA-DR – 75.0%, CD123 – 67.7%, CD64 – 48.5%, CD15 – 37.5%, CD65 – 31.3%, CD11b – 25.0%, CD61 -11.5%, CD14 – 7.3%, CD71 – 5.3% and Gly-A – 3.2%. Aberrant expressions of CD56 and CD7 were also found in 72.9% and 59.4% of the cases respectively. The application of 4-colour FCM had resulted in rapid and comprehensive characterisation of AML, leading to improvement in laboratory diagnostic precision and refinement of immunological classification of AML. This in turn permitted quick commencement of the most appropriate treatment strategy for the patients studied. In addition, unique antigen expression pattern in each case provided useful framework for disease monitoring as well as residual disease detection during and after treatment.

This research was supported by research grant no.: 02-01-09-SF0020.

IMMUNOPHENOTYPIC CHARACTERISATION OF ACUTE LYMPHOID LEUKAEMIA (ALL) IN SARAWAK USING 4-COLOUR FLOW CYTOMETRY

Researchers: *Researchers: Lela Suut[1], Tay Siow-Phing[1], Lau Lee-Gong[2], Mohamad Razif Othman[1], Ong Gek-Bee[3], Chew Lee-Ping[3] and Henry Rantai Gudum[1]*
[1]Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak
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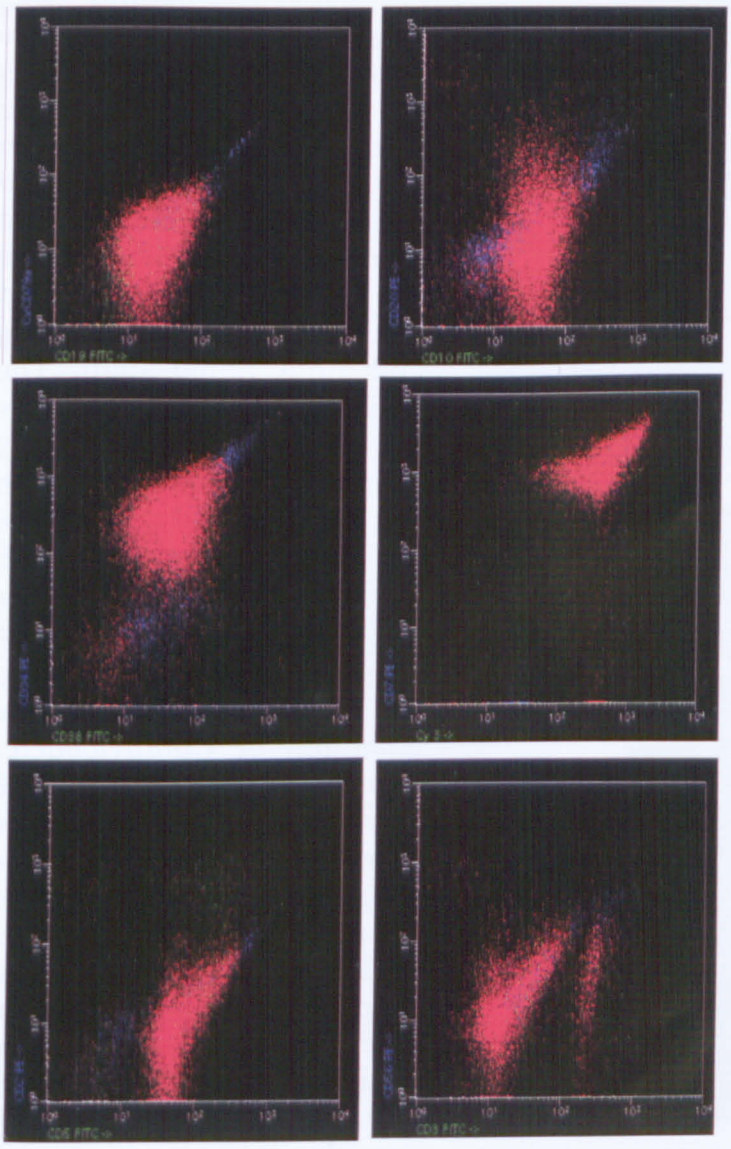


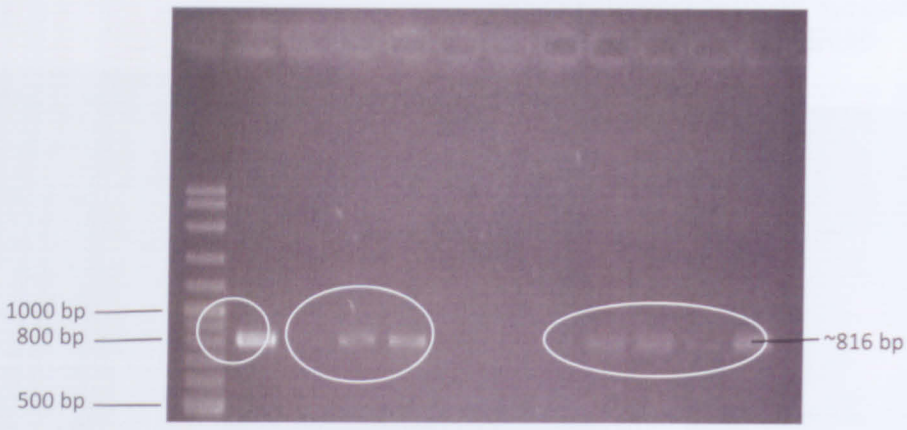
The identification of B-cell or T-cell leukaemic blasts (red) was based on the expressions of specific B-cell markers (e.g. cytoplasmic CD79a, CD19, CD10, CD20, CD38), or specific T-cell markers (e.g. cytoplasmic CD3, CD7, CD5, CD2, CD56).

Flow cytometric (FCM) investigation has become an important diagnostic tool in the diagnosis and lineage identification of acute lymphoblastic leukaemia (ALL). This study aimed to evaluate the immunophenotypic characteristics of the ALL cases diagnosed in Sarawak between 2008 to 2010. Bone marrow samples were analysed using 4-colour FCM that consisted of 6 and 7 tubes of markers for B-lineage and T-lineage respectively. There were 75 cases of ALL diagnosed within the study period, 86.7% were of B-lineage and the remainder (13.3%) were of T-lineage. Of these, 63.2% of the cases were ≤12 years old and the remaining (36.8%) were adults, with male to female ratio of 1.3:1. The B-ALL cases lacked of CD34 expression were 36.9%, whilst 52.3% CD45 was detected. All of the B-ALL cases expressed CD19, cytoplasmic CD79a and majority (92.3%) expressed CD22. Nuclear TdT expression and CD10 were detected in 87.7% and 83.1% of cases, respectively. Majority of the B-All cases were also positive for CD38 (87.7%). Only 18.5% and 10.8% of the B-ALL cases were positive for CD20 and HLA-DR. Lambda-chain restriction and cytoplasmic IgM was detected in a minority (<5.0%) of B-ALL cases. Only 10 cases of T-ALL were diagnosed during the period and all expressed CD7. Ninety percent of cases also expressed cytoplasmic CD3, nuclear TdT and CD5. The expression of other markers include: CD2 (80.0%); CD56 (70.0%); CD1a (20.0%), CD4 (30.0%); CD8 (40.0%), CD3 (40.0%) and HLA-DR (10.0%). Aberrant expression of CD13 was found in 40.0% of B-ALL cases and 20.0% in T-ALL. Another myeloid-associated marker, CD33 was detected in 35.4% of B-ALL cases and one case of T-ALL. 4-colour FCM had been proven to be useful in the identification of subtypes of ALL in Sarawak, and had facilitated diagnosis and initiation of treatment. Aberrant antigen expression may be useful in minimal residual disease study in these patients.

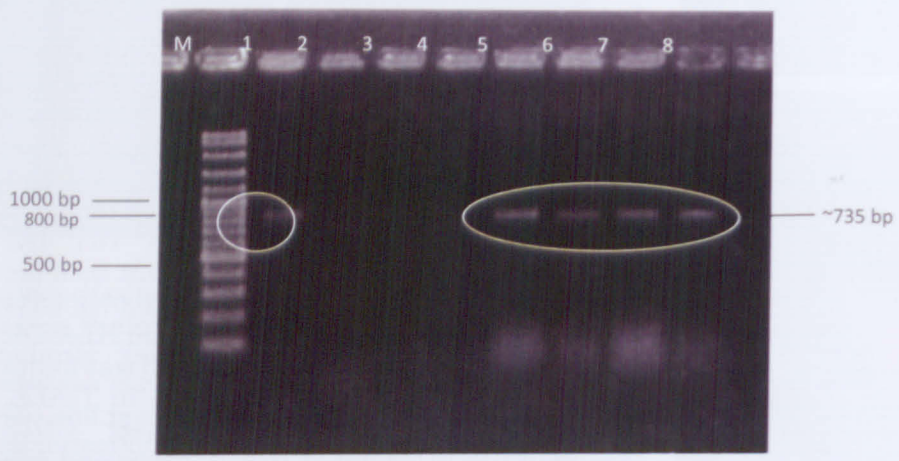
This research was supported by research grant no.: 02-01-09-SF0020.

DETECTION OF *CAMPYLOBACTER* SPP. AND *CAMPYLOBACTER JEJUNI* IN RAW VEGETABLES BY USING DIRECT PCR METHOD

Researchers: *Lesley Maurice Bilung, Adom Benjamen, Kasing Apun, Samuel Lihan and Micky Vincent*
Faculty of Resource and Science Technology, Universiti Malaysia Sarawak



Agarose gel electrophoresis of PCR products from vegetables samples for the detection of *Campylobacter* spp. on week 2. Lane M, DNA ladder of 100 kb. Lane 1, positive control of *Campylobacter jejuni* ATCC 33291. Lane 2, negative control. Lane 3 – 11, PCR products from vegetables samples. Lane 3 - 5, vegetables samples from Samarindah Market. Lane 3, Kai-lan. Lane 4, leaf mustard. Lane 5, lettuce. Lane 6 - 8, vegetables samples from Batu 7 Market. Lane 6, Kai-lan. Lane 7, leaf mustard. Lane 8, lettuce. Lane 9 - 11, vegetables samples from Stutong Community Market. Lane 9, Kai-lan. Lane 10, leaf mustard. Lane 11, lettuce.

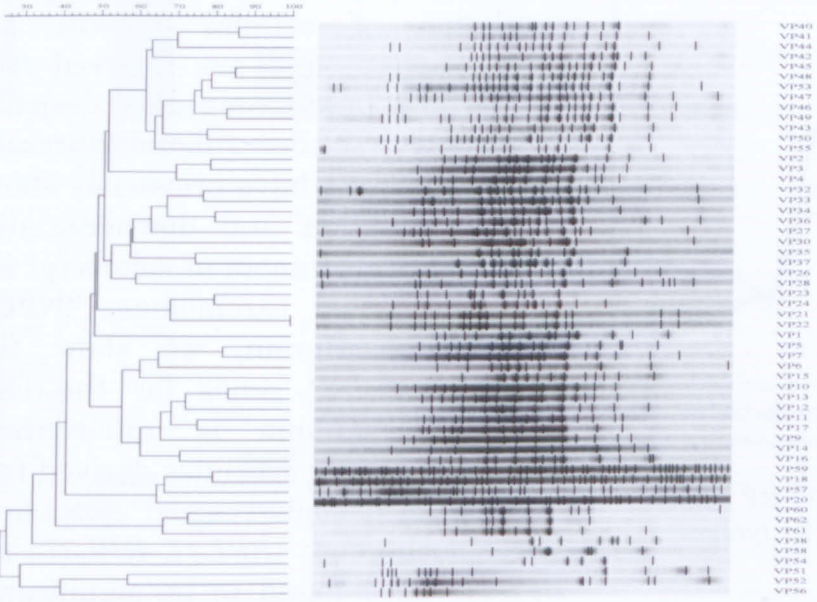


Agarose gel electrophoresis of PCR products from vegetables samples for the detection of *C. jejuni* on week 2. Lane M, DNA ladder of 100 kb. Lane 1, positive control. Lane 2, negative control of *Campylobacter jejuni* ATCC 33291. Lane 3 – 8, PCR products from vegetables samples. Lane 3 & 4, vegetables samples from Samarindah Market. Lane 3, Kai-lan. Lane 4, leaf mustard. Lane 5, lettuce from Batu 7 Market. Lane 6 – 8, vegetables samples from Stutong Community Market. Lane 6, Kai-Lan. Lane 7, leaf mustard. Lane 8, lettuce.

In this research, *Campylobacter* spp. and *Campylobacter jejuni* was detected from fresh and raw vegetable samples. Samples of three main vegetables namely, lettuce (*Lactuca sativa*), kai-lan (*Brassica alboglabra*) and leaf mustard (*Brassica chinensis*) were bought from three different wet markets in Kuching and Kota Samarahan. The three types of vegetables were bought randomly from three stalls in each wet market. In this study, Bolton Enrichment Broth Base (BEBB) was used as the enrichment media while Polymerase chain reaction (PCR) was used for the detection of *Campylobacter* spp. and *C. jejuni*. A total of 36 raw vegetables samples were subjected to PCR analysis targeting the *16S rRNA* gene for Campylobacter spp. and hip gene for *C. jejuni* respectively. Twelve out of thirty six samples (33%) were positive for *Campylobacter* spp. while four out of 36 samples (11%) were positive for *C. jejuni*. *Campylobacter* spp. and *C. jejuni* was successfully detected from the raw vegetables samples by using direct PCR method.

COMPARISON OF MOLECULAR TYPING OF *VIBRIO PARAHAEMOLYTICUS* ISOLATES USING RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) AND ENTEROBACTERIAL REPETITIVE INTERGENIC CONSENSUS – POLYMERASE CHAIN REACTION (ERIC-PCR) FINGERPRINTING

Researchers: *: Velnetti Linang, Lesley Maurice Bilung, Kasing Apun., Samuel Lihan and Micky Vincent*
Faculty of Resource Science and Technology, Universiti Malaysia Sarawak



Dendrogram showing ERIC-PCR profiles of typeable *V. parahaemoyticus* isolates.

A total of 62 *Vibrio parahaemolyticus* isolated from local cockles (*Anadara granosa*) collected from Tanjong Karang, Kuala Selangor were characterized using Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) and Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) assays. The RAPD analysis in this study revealed heterogeneous isolates of *V. parahaemolyticus* in cockles by using two primers (GEN1-50-03 and GEN1-50-04). Additionally, highly reproducible fingerprints for the isolates unique for each of the isolates were observed using ERIC-PCR. Results from this study demonstrated that genotyping *V. parahaemolyticus* isolates by using RAPD and ERIC-PCR is feasible for differentiation of various strains. Both ERIC-PCR and RAPD-PCR have shown to be rapid, sensitive and discriminative in typing the *V. parahaemolyticus* isolates from cockles.

DIFFERENTIAL EXPRESSION OF A SUBSET OF RIBOSOMAL PROTEIN GENES IN CELL LINES DERIVED FROM HUMAN NASOPHARYNGEAL EPITHELIUM

Researchers: *: Edmund Ui Hang Sim[1], Chow Hiang Ang[2], Ching Ching Ng[2], Choon Weng Lee[2], and Kumaran Narayanan[3]*

[1] Faculty of Resource Science and Technology, Universiti Malaysia Sarawak

[2]University of Malaya

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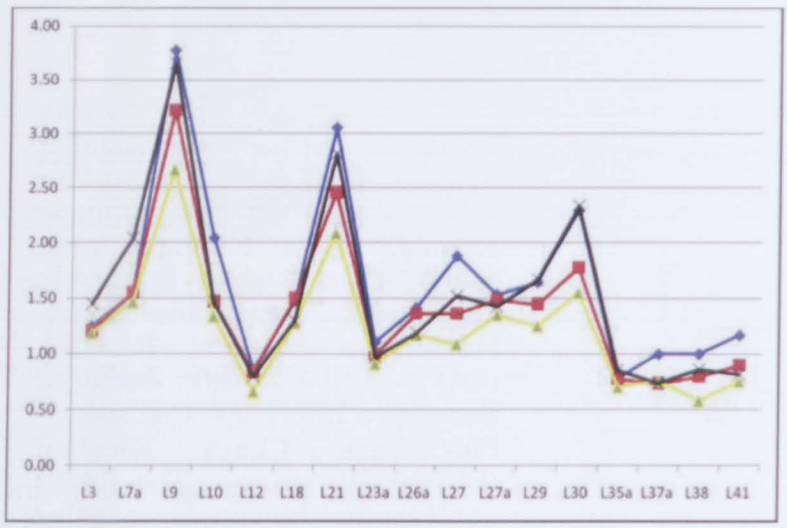

Line chart indicating expression profile of 17 RP genes in NP69 (blue line), TWO1 (red line), HONE1 (yellow line) and SUNE1 (black line) cell lines.

Extraribosomal functions of human ribosomal proteins (RPs) include the regulation of cellular growth and differentiation, and are inferred from studies that linked congenital disorders and cancer to the deregulated expression of RP genes. We have previously shown the upregulation and downregulation of RP genes in tumors of colorectal and nasopharyngeal carcinomas (NPCs), respectively. Herein, we show that a subset of RP genes for the large ribosomal subunit is differentially expressed among cell lines derived from the human nasopharyngeal epithelium. Three such genes (*RPL27, RPL37a* and *RPL41*) were found to be significantly downregulated in all cell lines derived from NPC tissues compared with a nonmalignant nasopharyngeal epithelial cell line. The expression of *RPL37a* and *RPL41* genes in human nasopharyngeal tissues has not been reported previously. Our findings support earlier suspicions on the existence of NPC-associated RP genes, and indicate their importance in human nasopharyngeal organogenesis.

This research was supported by research grant no.: IRPA-PR Topdown 06-02-09-1020-PR0054/05-02.

RELATIONSHIP OF ETHNICITY, AGE AND GENDER TO INCIDENCE OF BLADDER AND NASOPHARYNGEAL CANCERS IN KUCHING, SARAWAK

Researchers: *Edmund Ui-Hang Sim[1], Thung-Sing Tiong[1], Selva Kumar Subramaniam[2], Teng-Aik Ong[3], & Guan-Chou Teh[2]*

[1] Faculty of Resource Science and Technology, Universiti Malaysia Sarawak

[2]Sarawak General Hospital

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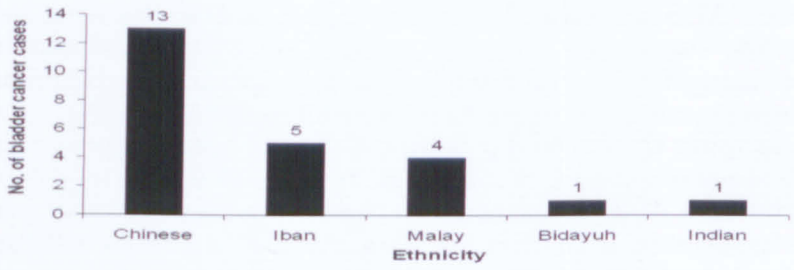

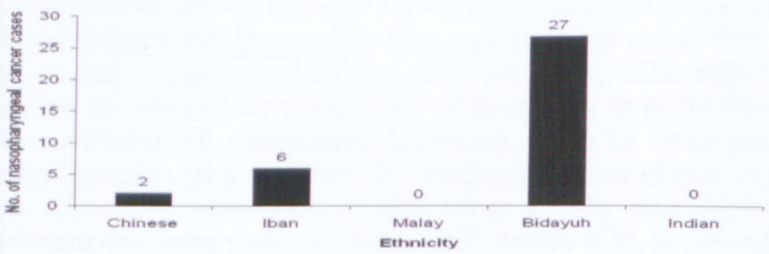

Records of cancer disease in Sarawak are semi-regular, with limited information available in the National Cancer Registry of 2003, and a more detailed record in the Sarawak Cancer Registry Report of 2005. These documents largely contain information on incidence and basic descriptive statistical account of age-standardised incidence rate for each type of cancer. Efforts to link incidence records of cancers in Sarawak with susceptibility factors such as ethnicity, gender and age are lacking and published reports of such studies are rare. In this paper, we present findings from the latest incidence analysis of bladder carcinoma (bladder cancer) and nasopharyngeal carcinoma (nose and throat cancer) in Kuching, and discuss probable linkage of incidence with ethnicity, gender and age. Our data provides useful baseline information for studies targeted at correlating non-environmental factors with bladder and nasopharyngeal cancers in the Kuching population of Sarawak.

This research was supported by research grant no.: IRPA-PR Topdown 06-02-09-1020-PR0054/05-02 and UNIMAS 01(120)/509/2005(08).

GENE EXPRESSION STUDIES OF HEART DISEASES IN THE SARAWAK POPULATION

Researchers: *Wen-Ni Tiong[1,2], Alan Yean-Yip Fong[2], Edmund Ui-Hang Sim[1], Sithy Harjieah Ibrahim[2], Kui-Hian Sim[2], Houng-Bang Liew[2], Seng-Keong Chua[2], Nor Hanim Mohd Amin[2], Choon-Kiat Ang[2], Kuan-Leong Yew[2], Tiong-Kiam Ong[2], Kim-Bee Lau[2], Annuar Rapaee[2]*

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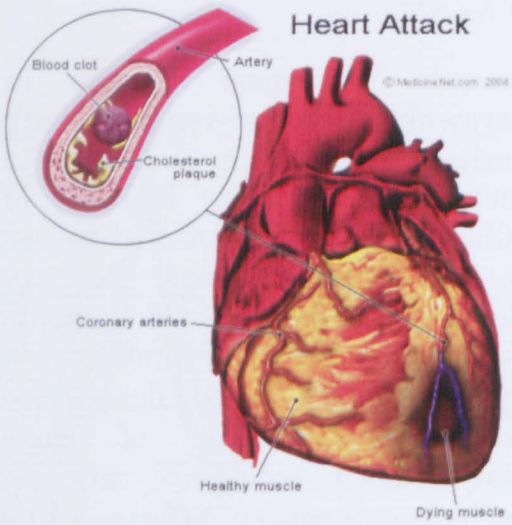
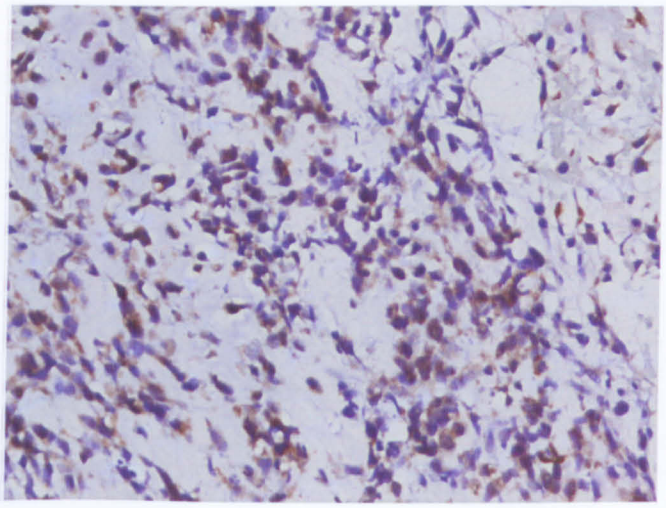


Acute Coronary Syndrome (ACS) commonly presents with the risk factors of hypertension, hyperlipidemia, diabetes and family history. However, there are many cases in which ACS patients do not have any of the classical risk factors, suggesting the presence of a yet unrevealed genetic predisposition or other pathological molecular mechanisms. Understanding these mechanisms and pathways is important to decipher the molecular manifestation of heart disease, from the stage of stable Coronary Artery Disease (CAD) into more significant ACS phenomena. In the search for genes that may be associated with this complex disease, a complementary approach of various gene expression profiling techniques can help to identify and validate clusters of relevant genes that may distinguish among different disease states. These genomic information, together with the traditional patient demographic and risk factor information, will enhance risk stratification in CAD and ACS patients, and may subsequently lead to the identification of new genetic biomarker molecules, as well as development of early detection strategies. In addition, the serum biochemical markers of various pathophysiological pathways also play a main role in diagnosis, prognosis and prediction of ACS event. This multi-marker strategy together with gene expression analyses can serve as a strong tool to characterize the exact underlying mechanisms of ACS, particularly in a multiethnic population in Sarawak who do seem to be admitted with ACS at a younger age when compared to the population in developed countries. Our studies examined whether patients with and without defined significant ACS can be distinguished by gene expression and protein markers assays on peripheral whole blood. Protein markers (established biochemical markers) were studied using enzyme immunosorbent assay (ELISA), while gene expression analyses were carried out using DNA microarray and quantitative (real time) PCR techniques. This landmark (pilot) study in Sarawak that involves multi-marker discovery will enable identification of genetic signatures associated with various pathological process of ACS. The molecular and biochemical characterization of ACS will provide information for future research on developing effective monitoring systems to diagnose the severity and progression of the disease.

This research was supported by research grant no.: NMRR-08-1418-2993.

VEGF EXPRESSION IN OSTEOSARCOMA IN SARAWAK GENERAL HOSPITAL

Researchers: *Mohammad Zulkarnaen Ahmad Narihan, Dayangku Norlida Awang Ojep, Zainal Abidin Rahim and Pan Kok Long*
Faculty of Medicine and Health Sciences, University Malaysia Sarawak



Immunohistochemical study using anti-VEGF antibody on osteosarcoma cases.

Angiogenesis has been shown to play a vital role in the progression of cancer. One of the most important angiogenic factors is the vascular endothelial growth factor (VEGF). High VEGF expression has been linked to poor outcome in many cancers: e.g. colorectal cancer, breast cancer, lung cancer. Research on angiogenesis in osteosarcoma have yielded conflicting results with some researchers reporting a poorer outcome in osteosarcoma patients with high angiogenesis while others report no relationship between the two. Other reports have noted a better outcome in patients with high angiogenesis expression who have undergone chemotherapy. We aimed to assess VEGF expression by immunohistochemical studies and correlate the expression with the clinical behaviour of osteosarcoma. A total of 28 patients were included in this study. Similar to previous studies, we did not find any significant correlation between VEGF positivity with age, gender, tumour size, and the tumour subtype. However, when assessing the survival rate of these patients, we found that the patients with VEGF positive tumours have a lower mean survival (16.9 months) than patients who have VEGF negative tumours (35.3 months) ($p = 0.011$). This suggests that the VEGF produced by OS may lead to a more aggressive tumour behaviour reflected in reduced mean survival of OS. This also suggests that VEGF tests may potentially be used as a marker to predict patient's outcome in osteosarcoma.

CYCLIN D1 OVEREXPRESSION IN ACRAL MELANOMA: A CASE CONTROL STUDY OF SARAWAKIAN PATIENTS

Researchers: *Zainal Abidin Ibrahim[1], M. Zulkarnaen A Narihan[1], Dk Norlida A Ojep[1], Ashley Edward Roy Soosay[1] and Jacqueline Wong Oy Leng[2]*
[1]Faculty of Medicine & Health Sciences, University Malaysia Sarawak
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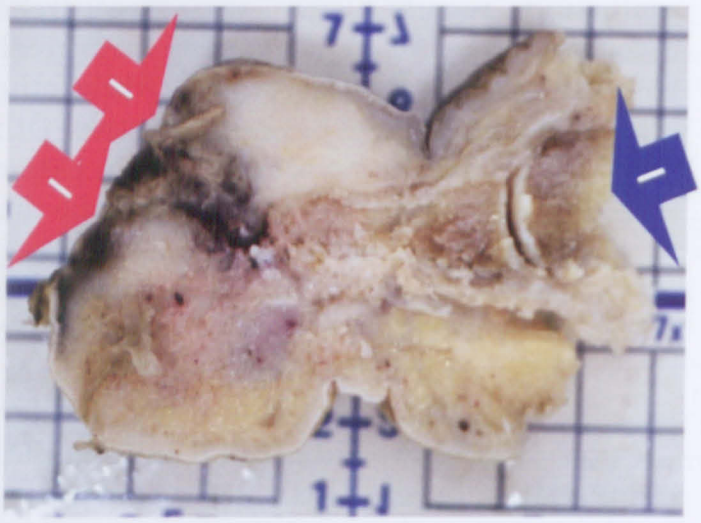
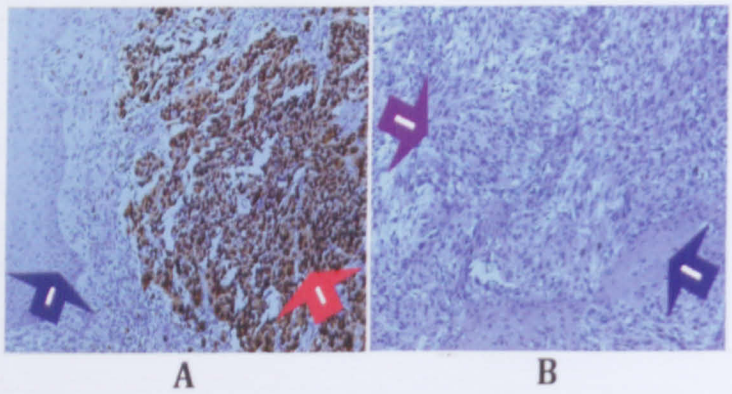
	Cyclin D1 overexpression status		X^2 value (df)	*p* value
	positive	negative		
Acral melanoma	19 (67.9%)	9 (32.1%)	4.721 (1)	<0.05
Non-acral melanoma	5 (33.3%)	10 (66.7%)		



Cut section of a big toe melanoma (red arrows). The lesion is an ulcerated tumour with pigmentation. Surgical margin at metatarsal bone (blue arrow).



A B

A: Cyclin D1 positive cells (red arrow); B: Cyclin D1 negative cells (purple arrow). The outer skin (blue arrows).

Acral melanoma (AM) is defined as malignan melanoma (MM) which occurs on the non-ha bearing skin of the palms and soles or unde the nail bed (Figure 1). It has distinctive clinica presentation and ethnic distribution compare to other subtypes of MM. This disease has high prevalence among Asians. It has bee characterized to exhibit distinctive focused gen amplification patterns. 11q13 is the commones amplified region which correlates with th cyclin D1 locus. Cyclin D1 overexpression wa reported in most AM cases in several studie among Caucasians. This finding has also bein observed in premalignant AM cells. This stud aimed to determine cyclin D1 overexpressio in Sarawakian cases of AM to improve th diagnosis and the treatment of the disease Cyclin D1 overexpression was assessed b immunohistochemistry method. Archiva paraffin-fixed tissues of MM were retrieved fron Department of Pathology, Sarawak Genera Hospital from 2004 until 2010. Twenty-eigh cases of AM were compared with 15 cases of non AM. Scores were recorded semi-quantitatively as follows (a) 0 if less than 10% of nuclei were stained (b) 1+, 10 to 25% of nuclei were stained (c) 2+, 25 to 50% of nuclei stained; (d) 3+, 50 t 75% of nuclei stained and (e) 4+ more than 75% of nuclei stained (Figure 2). Scores 1+ and above were regarded as positive. The AM cases exhibited significant cyclin D1 overexpression compared t non-AM cases ($p < 0.05$). This result is consistent with a similar study among Caucasian subjects reported by other researchers. Overexpression o cyclin D1 either contributes to the progressio past the G1-S phase of the cell cycle or acts as a pro survival factor. This finding helps to determine the adequacy of tumour resection in AM hence preventing recurrence, suggest the possible site of tumour primary in metastatic cases of MM and has the potential for targeted immunotherapy in treating AM. This finding significantly improves the accuracy of AM histological diagnosis and has the potential to enhance the management and treatment efficacy of this disease.

HUMAN PLASMODIUM KNOWLESI: DIAGNOSTICS AND OUTCOME

Researchers: *: Deshka Foster[1], Pek Peng Chin[2], Runggai Ketik[3], Sanjeev Krishna[1,4], Janet Cox-Singh[1,4] and Balbir Singh[1]*

[1]Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak

[2]Sarikei Hospital

[3]Sarikei Health Clinic

[4]St George's University of London

Deshka Foster, Fullbright US Student Researcher, evaluating a RDT.

Plasmodium knowlesi, a malaria parasite typically found in nature in long-tailed and pig-tailed macaques, is a cause of human malaria in Malaysia and in a number of other countries in Southeast Asia. Specific *Plasmodium* species are typically distinguished through microscopic examination of stained blood films. However there are a number of limitations of microscopy for the diagnosis of malaria, resulting in the development of alternative detection methods. Molecular detection assays are the most sensitive detection method for malaria and can distinguish *P. knowlesi* from the morphologically similar *P. malariae*. A number of rapid diagnostic tests (RDTs) for malaria have been described, mainly for the detection of *P. falciparum*, and have yet to be evaluated with blood samples from knowlesi malaria patients. Therefore a study was initiated to investigate the effectiveness of current RDTs in the detection of *P. knowlesi* infection. Preliminary findings indicate that the three RDTs that were evaluated for detection of knowlesi malaria were not as specific and sensitive as microscopy. Only those people who test positive for malaria by microscopy in Sarawak are admitted into hospital and treated. Consequently, it is suspected that a number of patients who have malaria, but are not diagnosed by microscopy, return to their communities with inappropriate treatment. It is unknown whether these people get worse and return to the health clinic or hospital later or if these infections self-cure. There is an ongoing study to accurately determine the number of people infected with *P. knowlesi* attending Health Clinics in Sarikei Division by molecular detection methods, and to determine the outcome of infections in people with malaria that were initially found to be malaria-negative by microscopy and were not hospitalized. Initial results indicate that there are cases of human *P. knowlesi*, which go undiagnosed due to malaria-negative microscopy results and that some of these patients appear to self-cure.

DIALYSIS CATHETER FIBRIN SHEATH STRIPPING: A USEFUL TECHNIQUE AFTER FAILED CATHETER EXCHANGE

Researchers: *Ahmad Faizal Mohamad Ali[1], Elna Uhwut [2] and Liew Shak Kui[2]*
[1]Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak
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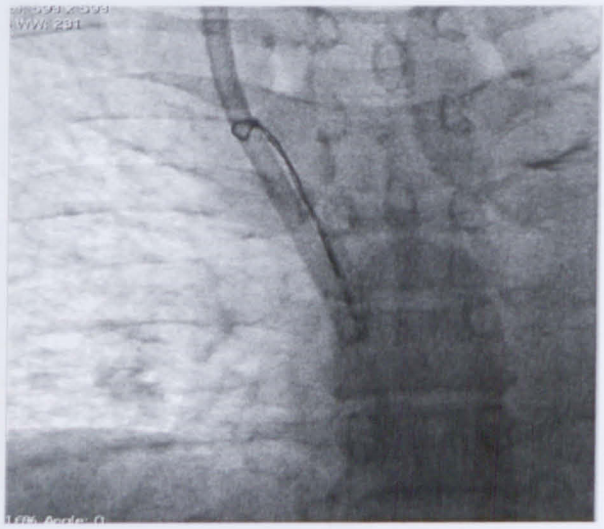

Fibrin sheath.

Tunneled dual lumen catheters are frequently used for long term haemodialysis access when arteriovenous fistulas or bypass grafts have failed or maturing. The most frequent reason for failing long term dialysis catheter is the formation of fibrin sheath. The term "fibrin sheath" is not completely accurate because the sheath can be composed of thrombus, endothelial cells and collagen depending on the duration of the catheter placement. The sheath covers the inlet and outlet holes of haemodialysis catheters acting as a one-way valve. Even partial encasement can prevent high flow rates required for satisfactory haemodialysis. Treatment options include pharmacologic and/or mechanical methods. Pharmacologic therapy involves instillation of urokinase (5,000 unit or above) or tissue plasminogen activator (2.5 mg in 50 mls normal saline over 3 hours) to lyses the thrombus. For mechanical treatment, an acceptable approach is catheter replacement over a guide wire. Other options include guide wire disruption, angioplasty balloon occlusion disruption and fibrin sheath stripping. A 60 year old man with end-stage renal failure on haemodialysis presented with failing dialysis access due to fibrin sheath formation. Initial 5,000 unit bolus of urokinase failed to improved the flow which was below 200 ml/min. A routine catheter exchanged was also unsuccessful. A method of stripping the fibrin sheath using a snare was performed and the catheter flow improved immediately. Six months later the flow remain above 300 ml/min. Fibrin sheath stripping is a good technique to restore catheter function after failed catheter exchanged with prolonged extended patency.

FLOW DIVERTERS: A NEW PARADIGM SHIFT IN MANAGEMENT OF COMPLEX CEREBRAL ANEURYSMS

Researchers: *Ahmad Faizal Mohamad Ali[1] and Ahmad Sobri Muda[2]*
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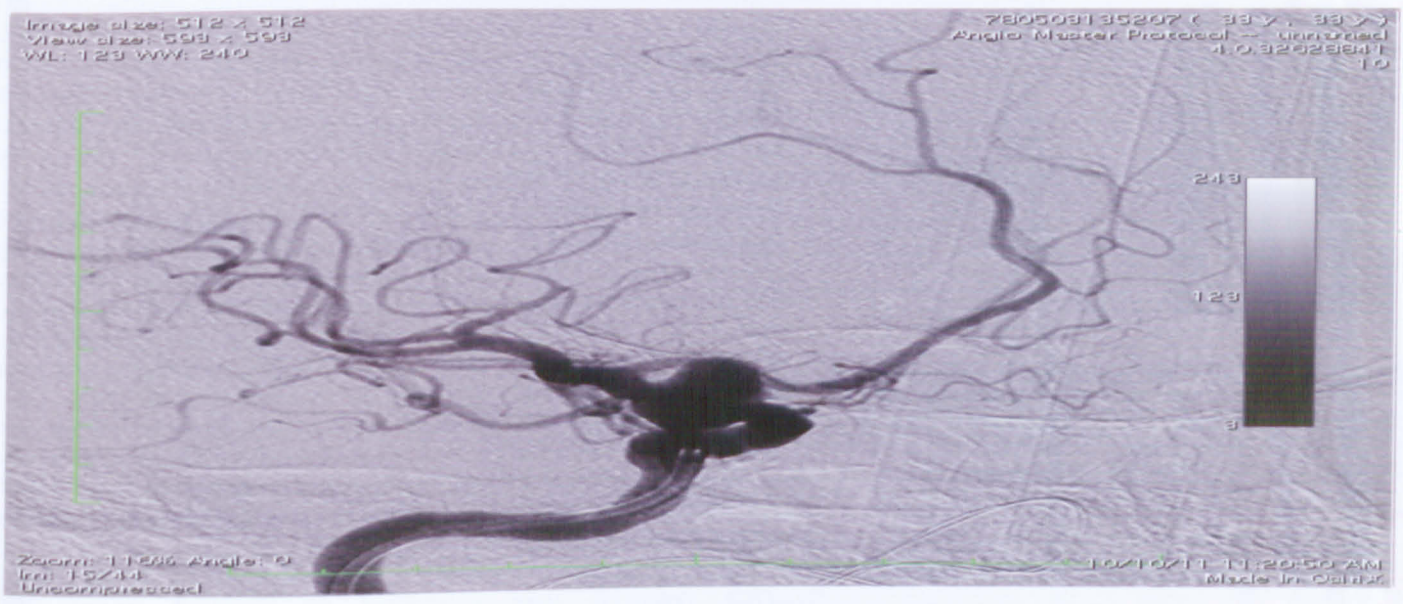
We present a case utilizing the new technique of parent vessel remodeling using SILK flow diverter (SFD) for a large, wide neck, fusiform-shape, supraclinoid aneurysm. The indications, benefits and technical aspect of the procedure are discussed. A 27 year-old technician presented with non-traumatic subarachnoid bleed. CT Angiography showed a large right supraclinoid internal carotid artery (ICA) aneurysm. Further detailed delineation with Digital Subtraction Angiography revealed a large, wide neck, fusiform aneurysm measuring 12 x 18 mm in diameter. The right anterior choroidal artery was seen arising from the dome of the aneurysm. After multidisciplinary team discussion, endovascular technique was judged as the best treatment approach. The advantages over surgical or other endovascular technique; the SFD offered less technical demand in its placement, sparing of important vessels arising from the aneurysm sac, minimum risk of rupture during procedure and early recovery. The unique ability of SFD in diverting blood flow is by virtue of its higher stent coverage (35-55%) in the parent vessel. Patient was started on dual anti-platelet therapy 10 days prior to the procedure and continued for 6 months to prevent in-stent thrombosis. Initial balloon occlusion test showed good cross over supply via the anterior and posterior communicating artery of The Circle of Willis. A scaffolding using Leo stent (Balt Extrusion, Montmorency, France) was placed across the aneurysm neck for added stability. A Vasco 21 microcatheter (Balt Extrusion, Montmorency, France) was placed beyond the aneurysm neck. The self-expanding SFD was slowly unsheathed and placed in the intended position. Post deployment angiogram showed stagnation of contrast in the aneurysm sac. SFD is a new concept in the treatment of intracranial aneurysm, which provide attractive alternative in instances where surgical, and aneurysm coiling were not feasible. Diverting the flow in the parent vessel will reduced the shear stress to the aneurysm wall. Overtime, progressive flow-reduction and stagnation will result in thrombosis. The added advantage is possibility of maintaining patency of vessels arising from the aneurysm sac, which would not attainable by other techniques.



Aneurysms.

ASSESSMENT OF LOCAL SPREAD FOR OSTEOSARCOMA AROUND THE KNEE

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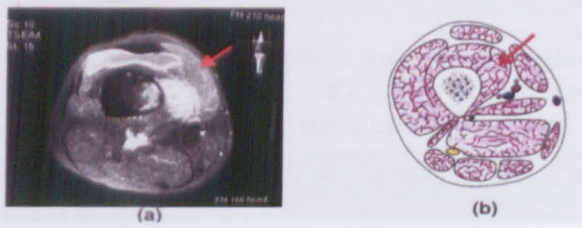
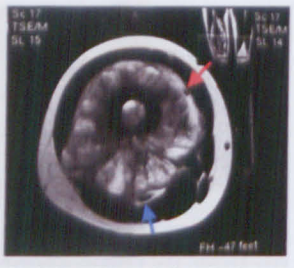
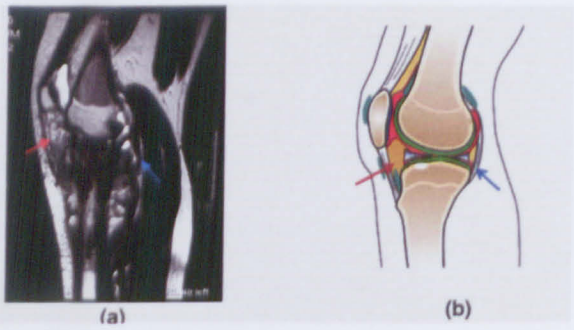


Axial fat suppressed gadolinium enhanced T1-weighted image showed a large disal femur osteosarcoma with infiltration to the vastus medialis (a). Cross sectional anatomy of the normal thight showing vastus medialis muscle (b).



Axial T2-weighted image showed a large distal femur osteosarcoma with infiltration ot the profunda femoral artery (blue arrow) and the femoral artery (red arrow).



Sagittal T2-weighted image showed a large proximal tibia osteosarcoma with infiltration to the infrapatellar fat (red arow) and posterior knee capsule (blue arrow) (a). Sagittal anatomy of the normal knee showing infrapatellar pad of fat and knee capsule (b).

Osteosarcomas are classically located in the bones around the knee. It typically presents in the distal femur and proximal tibia. However, the spread into the surrounding structures and tissues from the starting focal point has not been described. Contrasted MR (Magnetic Resonance) imaging allows exquisite visualization of the tumor in relation to the nascent anatomy (i.e. nerves, blood vessels, and muscles). In this study, we examined the sites of predilection of osteosarcoma in bones, muscles, neurovascular bundles and joints by using preoperative MR imaging. Furthermore, we investigated the correlation between histopathological subtype and the sites of predilection. The purpose of this study is to assess the sites of predilection for osteosarcoma around the knee in order to understand the local spread of the tumour and to assist in planning a limb salvage procedure. We examined preoperative MRI images in 16 patients with osteosarcoma around the knee to analyze the location, extension and infiltration of the tumour. The MRI findings were then correlated with histopathological findings. The primary lesions were at the metadiaphyseal region of femur, tibia and fibula. Involvement of epiphysis was encountered in a few cases. Medullary extent of the lesion is in the epiphysis and diaphysis. Extension to epiphysis usually involved the epiphyseal plate and sometimes involved the condyles of the femur and tibia. The extraosseous soft tissue infiltration was mainly at the medial side. The most common muscle involved was the vastus medialis. The other muscles involved were the vastus intermedius, semimembranosus, adductor magnus, popliteus, tibialis anterior and tibialis posterior. Medial femoral intermuscular septum, medial collectral ligament and medial patellar retinaculum were frequently infiltrated together with the vastus medialis muscle. Infiltration into neurovascular bundle was also found mainly at the medial side. The femoral nerve and vessels were most commonly infiltrated followed by profunda femoris vessels, popliteal vessels and sciatic nerve. Common involvement of the medial side of the knee may be due to weakness of the anatomic structures at this side. Among three vastus muscles, the first and most commonly infiltrated by the tumour was the vastus medialis. Joint invasion was found only in the tumour of the proximal tibia but the total number of cases assessed does not allow comment on whether this is significant. The proximal tibia is separated from knee joint and infrapatellar pad of fat only by a thin layer of cortex and periosteum. It is possible that this thin layer of bone represents an insufficient tissue barrier to tumour invasion into knee joint. No correlation was found between histopathological subtype and sites of predilection.

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